1926

MEMOIR 103 OCTOBER, 1926

CORNELL UNIVERSITY
AGRICULTURAL EXPERIMENT STATION

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THE GROWTH OF CERTAIN MICROORGANISMS IN PLANTED AND IN UNPLANTED SOIL

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ITHACA, NEW YORK
PUBLISHED BY THE UNIVERSITY
Received for publication June 18, 1926

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THE GROWTH OF CERTAIN MICROORGANISMS IN PLANTED AND IN UNPLANTED SOIL

J. K. Wilson and T. L. Lyon

A number of papers dealing with the influence of the living plant on certain bacterial processes in the soil have been published from this laboratory at various times. In common with other investigators we had observed what appeared to be an unaccountable disappearance of nitrates in soils on which certain crops grew. That nitrates disappear from soil on which crop plants are growing, and that the quantity of nitrogen absorbed by the crop is not always sufficient to account for all of the nitrate nitrogen that has disappeared, has now been demonstrated. The cause of this phenomenon has not been ascertained, but presumably the presence of the plant affects in some way the organisms that are active in the accumulation of nitrates.

If plant growth does influence the disappearance of nitrates in this way, it might be expected to affect the number of soil organisms concerned with nitrogen transformations. It was to ascertain the effect upon microbial population that the experiments here described were conducted.

Experiments dealing with this subject have usually approached it by a study of the products of bacterial action rather than by an enumeration of bacteria numbers. We have been able to find only two investigations in which the numbers of bacteria were determined concurrently in soil on which plants were growing and in similar unplanted soil. Hoffmann (1915) reports the results of experiments conducted by Hammer and Eldridge. These investigators drew samples of soil from near the roots of living plants and also at such a distance away as to avoid the presence of roots. Thirty-two tests were made, in which infusions of the soil were plated and the number of bacteria counted in the usual manner. On an average, two and one-half times as many bacteria were present in the samples taken near the roots as in those taken at a distance. Hoffmann's conclusion from this was that living plants influence the soil bacteria by changing the composition of the soil solution.

Joshi (1925), in studying the growth of root-nodule organisms of legumes, filled three flowerpots with Pusa soil. "Two legumes, sann-hemp and math, were grown in one. Two cereals, oats and maize, were grown in another. The third pot was left as control. When the crops were about two weeks old, samples of these soils were taken for plating. . . . . The

total number of colonies and of all organism colonies of the nodule organism in each plate were counted at the end of three and seven days' incubation at 30° C. and 20° C." Of the forty-eight platings there was only one in which the total number of colonies from the planted soil was less than that from the corresponding unplanted soil, and only one in which the nodule-organism colonies were less altho there were five cases of the latter in which the counts were the same. These were confined to the soil growing cereals, there being none in the legume-growing soil.

The hypothesis having been advanced that living plants influence nitrate accumulation by liberating from their roots organic matter which serves as a source of energy for nitrogen assimilation by soil organisms, it seemed to be desirable to study the effect of the living plant on the growth of certain microorganisms in pure cultures. The object in using pure cultures was to be sure that we were working with those organisms which are commonly supposed to produce transformations of nitrogen compounds. By inoculating a sterile medium with pure cultures, and comparing the rate of development of the organisms in the medium when plants were grown in it with the same phenomenon in the unplanted medium, the influence of the living plant might be ascertained. It is difficult to be certain that all the factors which may affect the growth of these organisms are the same in both the planted and the unplanted medium, but so far as possible the conditions, except those resulting from plant growth, were similar thruout.

METHODS OF PROCEDURE

Containers

Pyrex glass vessels 50 centimeters long and 6 centimeters in diameter, shaped like test tubes, had 300 grams of soil placed in each. This left sufficient space above the soil for the growth of the plants and also for the cotton plug which was used to prevent contamination. Over the top of the cotton and the tube was placed a sheet of paper which was held in place by a rubber band encircling the tube. The cotton and paper served not only as protection against contamination, but also to prevent rapid loss of water from the soil during the period in which the plants were growing.

Steaming and inoculation of soil

The tubes containing soil and cotton plugs were placed in an autoclave and heated for thirty minutes at a pressure of 25 pounds. During the steaming process the soil contained not more than 5 per cent of water, at which content less soluble matter is produced by steaming than is the case when the moisture content is greater. Since this is not enough moisture for the growth of plants, sterilized water containing the organism to be employed was added in quantity sufficient for inoculation and the moisture content was finally brought to 30 per cent of the dry weight of the soil.

From time to time, as the water evaporated from the tubes, it was replaced with sterile water. The moisture content was controlled by weighing the tubes, the weight and moisture of the original soil being known. After inoculation the tubes were stored for some time in order that the introduced organisms might reach their normal numbers before the seeds were planted in the soil.

Soils of two types were used, Dunkirk silty clay loam and Ontario loam. The former is a heavy soil having a rather high lime requirement; the latter, as implied by its class, is of medium lightness and usually shows by the Veitch method little or no lime requirement.

Planting the seed

The Wilson method of seed sterilization with calcium hypochlorite was used in preparing the seed for planting. After receiving the sterilizing treatment the seeds were taken from the solution and placed on the surface of the soil in the tubes. This was done by means of a long-handled spoon which could be readily sterilized by flaming. When maize was the plant to be used, only one kernel was planted in each tube; with timothy and red clover no attempt was made to secure uniform planting other than to estimate the number of seeds introduced, which ranged from 200 to 300.

Within three days after planting, both the tubes containing seeds and the corresponding tubes without seeds were taken to the greenhouse. In order to prevent the soil from becoming unduly hot in summer, the tubes were partly immersed in a tank thru which tap water was kept running constantly. During cold weather they stood in racks. At all times they were so placed that light and temperature were favorable for growth.

Removing the soil, and plating

The plants were allowed to grow for periods of various lengths ranging from a few weeks to more than five months. When the soil was to be removed and a comparison made between the planted and the unplanted soil, it was necessary to have tubes containing soil with the same moisture content. This was accomplished by weighing the tubes, by which means the moisture content could be calculated. In making these calculations no allowance was made for the weights of the plants. Usually several planted tubes and as many unplanted ones were opened at the same time. Those showing contamination when plated were discarded.

In order to measure the effect of plant growth on the number of organisms in the soil, the entire quantity of soil, together with the plants when these were present, was triturated with water in a large iron mortar. As rapidly as the soil became suspended in the water, the water was poured off and more was added. The process was repeated as often as necessary until all the soil was washed into one large container. The volume was then made up to 10 liters, and after being stirred thoroly a suitable quantity

was drawn for a determination of the number of organisms. A wide range of dilutions was made from the sample in order to secure a reliable count. A medium suitable for the growth of the organism or organisms was used. The incubation period, tho not of the same length in every case, was always sufficiently long to permit of the proper development of colonies before counting.

Since a number of different organisms were used, either singly or in combination, it was necessary to employ different media for plating. Ashby's medium was used for Azotobacter and for *Bacillus radicicola*; standard nutrient agar for *B. fluorescens*, the " nitrate-reducing organism," and the " guttation organism"; and soil-extract agar for the " blue organism." When combinations of organisms requiring different media were used, the plating was done on each of the suitable media.

Organisms used

Among the organisms with which the soil was inoculated were a few that are not well known. Some were used because they developed on the plates with unusual rapidity and for that reason could be counted before any associated organisms developed; others because the color of the colonies or some other easily distinguishing feature made it possible to identify them with certainty.

The " nitrate-reducing organism " was isolated from a laboratory solution of sodium nitrate in which part of the nitrate had been changed to nitrite. Colonies of the organism developed somewhat less rapidly on plates of standard nutrient agar than did the " guttation organism."

The " guttation organism " was isolated from water of guttation exuded from maize. It is an ammonifier, and developed colonies on the plates more rapidly than did any of the others.

The " blue organism " is an ammonifier isolated from soil on Caldwell Field. It forms characteristic blue colonies which are easily distinguishable.

Calculation of chances that results were not accidental

The method of Student has been used for calculating the significance of the results. Since counts were made of the colonies developed by plating soil infusions at various periods after planting, there are in all the experiments a sufficient number of cases on which to calculate the odds. This has been done for each experiment. The odds are stated at the bottom of each table. Odds of thirty or more to one may be taken to mean that the differences in the numbers of organisms in the plant-bearing and the plant-free soil were due to the difference in treatments. If we were successful in maintaining similar conditions in the soil of the tubes between which comparisons are made, the differences in numbers of organisms were due to the presence of living plants.

EXPERIMENT 1

Dunkirk silty clay loam was screened and used in the first experiment. It was heated and then inoculated with a pure culture of *B. radicicola* in the manner already described. An extra tube of soil was heated with the others and a determination was made of the nitrates. This showed the presence of 38 parts per million of NO_3 calculated to dry soil.

The tubes were heated, inoculated, and plugged on June 23, 1921, and were then allowed to stand in the laboratory until August 1, when they were planted with maize. Three days afterward they were taken to the greenhouse, partly immersed in running water, and allowed to stand until they were opened for plating. Two or more tubes were opened from time to time, when nitrates were again determined. Nitrates were always present in larger amounts in the unplanted soil than in the corresponding planted soil, but in no case were they absent. The results are shown in table 1. It will be noticed that the numbers varied considerably on the different dates. This is apparently to be accounted for by changes in the moisture content. Tubes with low moisture content gave low counts.

TABLE 1. GROWTH OF B. RADICICOLA IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	Date when organisms were counted	*B. radicicola*	
		Unplanted soil	Planted soil
1921	1921		
Sept. 23	Sept. 28	18.5	39.1
29	Oct. 5	4.8	45.4
Oct. 6	10	1.5	5.1
10	15	24.4	125.1
11	17	65.7	37.0*
17	24	9.7	41.2
17	24	5.7	19.6
25	31	13.6	114.3
25	31	4.6	49.5
27	Nov. 1	122.1	173.5
27	1	10.5	29.5
31	7	57.7	59.4
Nov. 11	16	11.2	53.1
Average		26.9	60.9

Odds 293 : 1

* The kernel of maize in this tube was moldy but no mold showed on the plates. The plant was not vigorous.

EXPERIMENT 2

The only respect in which a difference was intentionally instituted between experiments 1 and 2 was that in the latter calcium nitrate was added in solution in an amount sufficient to bring the nitrate content of the soil to 180 parts per million of NO_3 calculated to dry soil. This was done to insure the presence of a good supply of nitrate nitrogen, which it was thought might be reduced to a small quantity by heating in the autoclave. As the analyses of the unreinforced soil after heating and when the tubes were opened dispelled this fear, the practice was not continued except in experiment 4. The dates of heating, inoculating, and planting the soil were the same as in experiment 1. The results of the platings of experiment 2 are given in table 2:

TABLE 2. GROWTH OF B. RADICICOLA IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	Date when organisms were counted	*B. radicicola*	
		Unplanted soil	Planted soil
1921	1921		
Sept. 23	Sept. 28	2.8	41.5
29	Oct. 5	2.3	11.7
Oct. 6	10	1.6	24.8
10	15	14.0	17.9
11	17	12.3	8.0
17	24	4.1	35.0
17	24	3.7	11.6
25	31	14.2	94.0
25	31	5.5	6.8
27	Nov. 1	2.6	17.0
27	1	31.1	42.6
Average		8.6	28.3

Odds 83.7 : 1

EXPERIMENTS 3 AND 4

The same type of soil was used in experiments 3 and 4 as in experiments 1 and 2, and the same kind of plant was grown, but the organism used for inoculation was different. The name "nitrate-reducing organism" was employed for want of a better one. The organism was isolated from a dilute stock solution of sodium nitrate which was found to be losing strength owing apparently to the presence of this bacterium. It proved itself an energetic agent for converting nitrates into nitrites.

In experiment 3 no calcium nitrate was added to the soil, but in experiment 4 the content of NO_3 was brought up to about 180 parts per million of dry soil. There seemed to be nothing to gain by adding calcium nitrate and the practice was discontinued after these two trials. In both experiments the soil was heated and inoculated on February 28, 1921, and planted on April 6 of the same year. The results from experiments 3 and 4 may be found in tables 3 and 4, respectively.

TABLE 3. GROWTH OF "NITRATE-REDUCING ORGANISM" IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	Date when organisms were counted	"Nitrate-reducing organism"	
		Unplanted soil	Planted soil
1921	1921		
April 29	May 2	67.3	114.4
May 2	5	92.0	98.2
6	9	88.8	104.8
9	13	95.2	140.7
11	16	107.2	162.2
14	17	107.1	192.7
17	20	111.2	190.1
20	23	105.9	226.9
23	26	95.2	153.1
June 3	June 6	93.4	215.7
7	10	122.6	265.6
10	13	194.5	235.0
14	17	125.2	197.3
Average		108.1	176.7

Odds 9999+ : 1

TABLE 4. GROWTH OF "NITRATE-REDUCING ORGANISM" IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	Date when organisms were counted	"Nitrate-reducing organism"	
		Unplanted soil	Planted soil
1921	1921		
April 29	May 2	66.8	65.0
May 2	5	107.6	102.2
6	9	111.5	134.2
9	13	119.1	113.4
11	16	38.1	135.0
14	17	116.8	176.4
17	20	129.5	184.3
20	23	97.5	162.0
23	26	178.0	165.2
June 14	17	59.6	144.7
18	21	67.0	114.3
18	21	61.2	107.9
Average		96.0	133.7

Odds 369 : 1

EXPERIMENT 5

The respect in which experiment 5 differs from the previous ones is in the use of *Pseudomonas fluorescens* as the inoculant. It proved to be a convenient organism with which to work, because of the very distinctive character of the colonies formed. The soil was heated and inoculated on October 30, 1921, and planted on November 30. The results are presented in table 5:

TABLE 5. GROWTH OF PS. FLUORESCENS IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	Date when organisms were counted	*Ps. fluorescens*	
		Unplanted soil	Planted soil
1922	1922		
Jan. 10	Jan. 16	0.2	0.6
12	19	5.0	14.5
Feb. 2	Feb. 6	6.7	8.7
7	13	1.2	4.4
16	20	2.6	11.7
21	24	3.3	17.3
March 2	March 6	0.4	9.8
2	6	3.2	3.4
Average		2.8	8.8

Odds 151:1

EXPERIMENT 6

In experiment 6, inoculation was performed with an organism isolated from water of guttation obtained from maize plants. This organism formed colonies on the agar more quickly than did any of the other organisms, and could be easily recognized. It is a vigorous consumer of nitrates. The soil was heated and inoculated on January 27, 1922, and planted on March 1. The results of the test are reported in table 6:

TABLE 6. GROWTH OF "GUTTATION ORGANISM" IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	Date when organisms were counted	"Guttation organism"	
		Unplanted soil	Planted soil
1922	1922		
April 4	April 6	26.1	39.6
8	10	37.6	37.6
11	13	17.5	33.9
13	15	10.3	35.6
16	19	0.7	35.0
21	24	13.1	91.7
25	27	12.5	43.1
May 2	May 4	24.3	30.4
2	4	7.9	21.0
2	4	9.1	28.4
2	4	10.6	23.1
2	4	0.8	17.2
Average		14.2	36.4
Odds 666:1			

EXPERIMENT 7

A combination of two organisms was used in experiment 7, the two being the " nitrate-reducing organism " and *B. radicicola*. Both developed more numerously in the planted soil than in the unplanted soil, as may be seen in table 7. The soil was heated and inoculated on October 29, 1921, and planted on November 30. The results are given in table 7:

TABLE 7. GROWTH OF " NITRATE-REDUCING ORGANISM " AND B. RADICICOLA IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	Date when organisms were counted	"Nitrate-reducing organism"		*B. radicicola*	
		Unplanted	Planted	Unplanted	Planted
1922	1922				
Jan. 27	Jan. 31	25.6	35.8	40.6	41.6
Feb. 17	Feb. 23	9.1	23.0	42.2	59.0
23	28	44.2	59.1	25.1	44.1
March 20	March 25	6.6	18.6	12.4	20.9
April 4	April 8	52.8	103.3	87.1	148.5
8	11	58.1	124.2	4.6	8.6
12	15	35.6	106.3	43.6	112.2
13	16	15.7	47.0	20.3	133.6
17	21	34.0	135.3	4.1	80.5
21	24	17.7	89.1	17.7	33.3
25	29	56.1	158.4	14.5	41.2
May 4	May 8	6.9	71.8	15.8	82.5
21	24	10.4	133.6	0.5	5.8
22	27	32.9	85.8	0.7	7.0
26	29	11.9	77.6	3.0	7.6
	Average	27.8	84.6	22.1	55.1

Odds for "nitrate-reducing organism" 9999+ :1
Odds for *B. radicicola* 908:1

EXPERIMENT 8

The number of organisms was increased to three in experiment 8. They were the "nitrate-reducing organism," the "blue organism," and *B. radicicola*. As in experiment 7 all three organisms made greater growth in the planted soil than in the unplanted soil. The soil was heated and inoculated on May 30, 1922, and planted on February 14, 1923. The results are presented in table 8:

TABLE 8. GROWTH OF "NITRATE-REDUCING ORGANISM," BLUE ORGANISM," AND B. RADICICOLA, IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	"Nitrate-reducing organism"		"Blue organism"		*B. radicicola*	
	Unplanted soil	Planted soil	Unplanted soil	Planted soil	Unplanted soil	Planted soil
1923						
March 3	4.0	23.4	5.3	14.7	4.3	8.9
April 18	7.3	37.3	7.3	41.4	8.6	21.0
18	19.5	19.8	23.1	24.7	20.8	21.2
May 10	12.5	14.8	1.0	1.5	16.2	19.8
10	16.5	24.1	10.6	46.2	8.9	34.3
11	14.0	29.9	8.7	57.7	10.6	29.7
11	5.9	7.1	14.2	18.6	18.0	18.0
June 5	19.3	25.1	85.8	85.8	57.4	60.2
27	15.7	52.8	8.7	72.3	6.6	77.2
Average	12.7	26.0	18.3	40.3	16.8	32.3

Odds for "nitrate-reducing organism" 108:1
Odds for "blue organism" 69:1
Odds for *B. radicicola* 28:1

EXPERIMENT 9

All the preceding experiments had been conducted with maize, a plant that grew quickly and vigorously in the tubes. A number of experiments were later undertaken in which timothy grass (*Phleum pratense*) was used. The soil type was the same as that employed in the previous experiments. In experiment 9 the organism with which the soil was inoculated was the "nitrate-reducing organism." The soil was heated and inoculated on November 17, 1921, and planted on December 3. The results of the experiment are given in table 9:

TABLE 9. GROWTH OF "NITRATE-REDUCING ORGANISM" IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO TIMOTHY

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	Date when organisms were counted	"Nitrate-reducing organism"	
		Unplanted soil	Planted soil
1922	1922		
Jan. 31	Feb. 3	31.9	79.5
Feb. 25	28	24.8	116.7
April 4	April 7	108.9	176.9
18	22	48.8	242.2
May 2	May 6	17.5	227.7
2	6	36.8	173.2
20	24	72.6	321.7
21	26	10.6	283.8
22	26	72.6	153.4
22	26	122.4	550.1
26	30	21.3	130.3
Average		51.6	223.2

Odds 3332 : 1

EXPERIMENT 10

Timothy was again used in experiment 10. The organism was *Ps. fluorescens*. The soil was the same as in the previous experiments. The soil was heated and inoculated on March 17, 1922, and planted on April 3. The results are given in table 10:

TABLE 10. Growth of Ps. fluorescens in Dunkirk Silty Clay Loam Unplanted and Planted to Timothy

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	Date when organisms were counted	*Ps. fluorescens*	
		Unplanted soil	Planted soil
1922	1922		
May 19	May 22	7.6	16.8
19	22	2.3	41.4
26	29	1.3	13.2
June 10	June 13	0.5	21.0
10	13	17.5	33.1
20	23	2.4	181.5
21	26	0.7	39.0
30	July 5	4.4	64.7
30	5	69.3	103.9
Average		11.8	57.2

Odds 57:1

EXPERIMENT 11

Three organisms were used in experiment 11. They were the "nitrate-reducing organism," the "blue organism," and *B. radicicola*. The soil was heated on February 20, 1923, inoculated on February 27, and planted to timothy on April 6. The results are shown in table 11:

TABLE 11. GROWTH OF "NITRATE-REDUCING ORGANISM," "BLUE ORGANISM," AND B. RADICICOLA, IN DUNKIRK SILTY CLAY LOAM UNPLANTED AND PLANTED TO TIMOTHY

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	"Nitrate-reducing organism"		"Blue organism"		*B. radicicola*	
	Unplanted soil	Planted soil	Unplanted soil	Planted soil	Unplanted soil	Planted soil
1923						
April 19	9.9	146.8	9.7	297.6	7.9	225.7
21	13.4	179.8	9.1	207.9	11.7	153.4
24	32.7	125.4	4.8	54.1	44.4	176.6
28	10.6	151.8	10.2	171.6	9.4	268.9
28	22.8	169.9	29.7	273.6	28.9	188.1
May 15	25.1	120.4	31.2	153.4	18.8	110.5
15	18.3	68.5	20.1	100.6	13.4	51.1
June 11	4.9	53.8	....	....	3.1	72.6
27	14.2	96.4	13.9	184.8	15.2	201.3
July 17	5.8	132.0	9.4	107.2	10.6	143.5
Average	15.8	124.5	15.3	172.3	16.3	159.2

Odds for "nitrate-reducing organism" 9999+:1
Odds for "blue organism" 4999:1
Odds for *B. radicicola* 9999+:1

EXPERIMENT 12

In experiment 12 the soil was inoculated with *Azotabacter chroöcoccum*, but since it had been found in previous work that Azotobacter does not grow readily in Dunkirk silty clay loam unless limed, enough calcium carbonate was added to satisfy the lime requirement. The soil was heated on February 20, 1923, inoculated on February 27, and planted on April 6. Timothy was again the crop grown. The results are given in table 12:

TABLE 12. GROWTH OF AZOTOBACTER CHROÖCOCCUM IN DUNKIRK SILTY CLAY LOAM WITH $CaCO_3$ ADDED, WHEN UNPLANTED AND WHEN PLANTED TO TIMOTHY

(The figures indicate thousands of organisms per gram of soil)

Date of opening tubes	*Azotobacter chroöcoccum*	
	Unplanted soil	Planted soil
1923		
April 19	22.8	32.2
20	17.8	26.4
20	11.3	13.5
21	18.3	21.1
24	4.0	13.9
25	27.2	38.3
May 1	10.7	66.0
1	10.2	24.1
2	11.2	17.8
2	11.5	14.8
5	11.5	27.1
5	22.4	26.4
16	8.3	17.3
16	8.4	13.9
16	19.5	21.6
16	14.2	21.0
22	33.0	34.8
23	15.0	66.8
24	17.5	26.7
June 5	9.6	13.5
11	9.2	13.4
Average	14.9	26.2
Odds	1110 :1	

It will be noticed, in comparing tables 9, 10, 11, and 12, that the numbers of organisms were relatively much greater under the timothy than under the maize when both are compared with the corresponding unplanted soil. This may mean that timothy is more effective than maize in furthering the growth of the organisms used in the experiment, but as the incubations

were not made at the same time it would not be entirely safe to draw that conclusion.

EXPERIMENT 13

It was decided to ascertain what the effect would be if Dunkirk silty clay loam were limed and one of the organisms other than Azotobacter were used in the test. The "nitrate-reducing organism" was used in experiment 13, and at the same time the soil was inoculated with Azotobacter. Calcium carbonate was added to the soil in the same quantity as before. The growth of the "nitrate-reducing organism" in the unplanted and in the planted soil was similar to its growth in the unlimed soil as regards the effect of the growing plants. The soil was heated and inoculated on June 19, 1923, and planted on October 15. The results are given in table 13:

TABLE 13. GROWTH OF "NITRATE-REDUCING ORGANISM" AND AZOTOBACTER CHROÖCOCCUM IN DUNKIRK SILTY CLAY LOAM WITH $CaCO_3$ ADDED, WHEN UNPLANTED AND WHEN PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	"Nitrate-reducing organism"		*Azotobacter chroöcoccum*	
	Unplanted soil	Planted soil	Unplanted soil	Planted soil
1923				
Nov. 21	379.5	541.7	72.6	97.7
30	379.5	448.8	59.4	82.5
30	379.5	420.7	59.4	120.8
Dec. 4	320.4	420.7	4.4	6.0
4	320.4	363.0	4.4	5.2
21	244.7	422.4	53.5	75.9
21	298.6	430.6	60.9	95.0
Average	331.8	435.4	44.9	69.0

Odds for "nitrate-reducing organism" 713:1
Odds for Azotobacter 87:1

EXPERIMENT 14

The experiments with Dunkirk silty clay loam had in almost all cases resulted in a greater growth of organisms in the planted soil than in the corresponding unplanted soil. In experiment 14 and the remainder of the experiments reported in this paper, Ontario loam was used. This is a soil found rather widely distributed in New York and is considered to be very productive. It does not usually have any lime requirement altho in some sections it shows need of small quantities of lime. The "guttation organism" was used in this experiment. The soil was heated and inoculated on June 19, 1923, and planted on October 15. The results are presented in table 14:

TABLE 14. Growth of "Guttation Organism" in Ontario Loam Unplanted and Planted to Maize

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	"Guttation organism"	
	Unplanted soil	Planted soil
1923		
Oct. 25	37.3	49.5
29	55.4	86.6
Nov. 1	5.6	9.1
1	2.7	2.7
15	44.0	84.5
15	32.2	90.6
15	22.9	50.8
15	44.4	66.0
20	34.3	64.0
Average	31.0	56.0
Odds 555:1		

EXPERIMENT 15

Ontario loam was again used in experiment 15, but it was limed with calcium carbonate to meet the lime requirement. It was sterilized on June 23, 1923, and subsequently inoculated with Azotobacter. Maize was planted on September 26. The results are given in table 15:

TABLE 15. GROWTH OF AZOTOBACTER CHROÖCOCCUM IN ONTARIO LOAM WITH $CaCO_3$ ADDED, WHEN UNPLANTED AND WHEN PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	*Azotobacter chroöcoccum*	
	Unplanted soil	Planted soil
1924		
Jan. 2	1.3	3.0
2	1.3	1.6
2	1.3	2.9
April 11	2.8	7.7
May 18	3.2	5.8
18	3.2	4.8
18	3.2	6.3
June 18	0.1	0.6
18	0.1	0.3
18	0.1	0.3
18	0.1	6.9
30	0.2	2.2
30	0.5	1.2
30	0.5	1.1
30	0.5	1.4
Average	1.2	3.1
Odds	908:1	

EXPERIMENT 16

It was thought desirable to conduct one or two additional experiments with Ontario loam. Since previous experiments had shown that it was possible to inoculate with several organisms at the same time and make satisfactory platings, the "nitrate-reducing organism," the "blue organism," and *B. radicicola* were used in experiment 16. The soil was heated and inoculated on May 2, 1922, and planted on February 14, 1923. The results, which have the same general trend as for the experiments conducted with maize on Dunkirk silty clay loam, are recorded in table 16:

TABLE 16. GROWTH OF "NITRATE-REDUCING ORGANISM," "BLUE ORGANISM," AND B. RADICICOLA, IN ONTARIO LOAM UNPLANTED AND PLANTED TO MAIZE

(The figures indicate millions of organisms per gram of soil)

Date of opening tubes	"Nitrate-reducing organism"		"Blue organism"		*B. radicicola*	
	Unplanted soil	Planted soil	Unplanted soil	Planted soil	Unplanted soil	Planted soil
1923						
March 31	4.0	23.4	5.3	14.7	4.3	8.9
May 10	12.5	14.8	1.0	1.5	16.2	19.8
10	16.5	24.1	10.5	46.2	8.9	34.3
11	14.0	29.8	8.7	57.7	10.6	29.7
11	5.9	7.1	14.2	18.6	17.9	17.9
June 5	19.3	25.1	85.8	85.8	57.4	60.2
27	15.7	52.8	8.7	72.2	6.6	77.2
Average	12.6	25.3	19.2	42.4	17.4	35.4

Odds for "nitrate-reducing organism" 55:1
Odds for "blue organism" 32:1
Odds for *B. radicicola* 16:1

EXPERIMENT 17

In order to determine whether the seeds alone might furnish energy for the development of the organisms with which the soil was inoculated, a special experiment was conducted. Tubes of soil were prepared as in the other experiments and otherwise treated in the same manner except that the seeds were devitalized before being placed in the tubes. The tubes were opened and soil infusions plated four weeks after the seed was planted. As in the other experiments, a tube containing seed and one without seed was opened and plated at the same time. The seed used was timothy. The "nitrate-reducing organism" was used to inoculate the soil. The results of the counts are presented in table 17:

TABLE 17. EFFECT OF DEAD SEED ON BACTERIA NUMBERS IN DUNKIRK SOIL, "NITRATE-REDUCING ORGANISM"

(The figures indicate millions of organisms per gram of soil)

Set no.	Without seed	With seed
1	229.6	216.4
2	312.0	328.3
3	16.2	13.7
4	7.5	20.0
5	207.6	210.0
6	96.2	79.2
7	109.2	110.4
8	84.0	78.0
9	90.2	94.0
10	132.0	154.8
Average	128.4	130.5
Odds 2.6:1		

The figures in table 17 show very little difference between the tubes with seed and those without, in respect to the number of organisms. There appears to be little if any influence of the dead seed upon the number of the "nitrate-reducing organisms" present.

DISCUSSION

The experiments herein described represent another step in the investigation of the reason for the unexplained disappearance of nitrate in soil on which certain crops are growing. Working on the hypothesis that living plants liberate from their roots organic matter which furnishes additional energy for soil organisms, the writers conducted experiments a few years ago with maize and some other crop plants, in sterile nutrient solutions, and found that organic substances are liberated (Lyon and Wilson, 1921). The organic matter liberated contained only a small proportion of nitrogen. It might, therefore, be expected to furnish energy for soil microorganisms.

The present experiments demonstrate a greater development of certain soil organisms where plants are growing than where they are not. It has not been proved that the greater microbial population resulted from the utilization by the microbes of the substances liberated. This is, however, a rather natural supposition.

An investigation by Neller (1922) tends to support the hypothesis in question. He found, by means of an ingenious device, a more abundant formation of carbon dioxide in soil in which buckwheat, barley, soybeans, or field peas were growing, than in unplanted soil. A greater production of carbon dioxide might be expected to accompany an increased development of soil organisms. In fact, Wilson and Wilson (1925) found that the evolution of carbon dioxide in soil inoculated with certain ammonifying organisms was directly proportional to the number of these organisms present, and further that this evolution of carbon dioxide is in inverse order to the quantity of nitrate nitrogen present. These investigators' results were obtained when plant tissues in process of decay were present in the soil. As decomposition of the organic matter started, nitrates began to disappear, the evolution of carbon dioxide increased, and with this increase the number of ammonifying organisms became greater.

These three phenomena have been found to proceed in the same order when higher plants are growing on soil. Since certain plants have been found to liberate organic matter from their roots, it seems reasonable to believe that it is this organic matter which causes the increase in the numbers of microorganisms, that these organisms increase the evolution of carbon dioxide in the soil, and that by their consumption of nitrate nitrogen they cause a disappearance of nitrates not absorbed by the plants growing on the soil.

SUMMARY

Soil was placed in large tubes, and after these had been heated in an autoclave and inoculated with pure cultures of selected organisms, sterilized seeds of maize or timothy were planted in some of the tubes while others remained unplanted.

After the plants had grown for periods varying from several days to a few months, the soil was removed from both the planted and the unplanted tubes, dilute infusions were plated, and colonies were counted after incubation.

With few exceptions the soil in which plants were growing contained a larger number of organisms than did the unplanted soil. The organisms used were in every case those known to be active in changing nitrogen from one form to another.

The conclusion is drawn that at least some crop plants produce a condition in the soil surrounding their roots which favors the growth of certain microorganisms that consume nitrate nitrogen, thus accounting for the disappearance of nitrate nitrogen not absorbed by the plants.

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